REMARKS

Claims 1-6, 9-14 and 17-19 were pending in the present application. Claims 4 and 5 have been amended to change their dependency. No new matter has been added.

Amendments to the claims should in no way be construed as an acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s). Attached hereto is a marked-up version of the changes made to the claims by the current amendments.

Double Patenting Rejection of Claims 1-6, 9-14, and 17-19

Claims 1-6, 9-14, and 17-19 are rejected under the judicially created doctrine of nonstatutory obvious-type double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 5,888,981. Applicants will address this issue upon an indication of allowable subject matter.

Rejection of Claims 1-6 and 9 under 35 U.S.C. § 112, First Paragraph

Claims 1-6 and 9 are rejected under 35 U.S.C. § 112, first paragraph, because the Examiner asserts that the specification does not enable one of ordinary skill in the art to practice the invention.

Claims 4 and 5 have been amended in order to expedite prosecution of the present application. Applicants reserve the right to pursue claims 4 and 5 as originally filed in this or a separate application(s).

The Examiner acknowledges that the specification is enabled for certain methods of regulating expression of a *tet* operator-linked gene in a cell, wherein a first nucleic acid molecule comprises a nucleic acid molecule comprising the *tet* operator-linked gene, and a second nucleic acid molecule encodes a tetracycline-controllable transactivator (tTA), wherein the tTA comprises a Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eukaryotic cells. The Examiner states that the above-mentioned method of regulation is enabled when:

1. the method is carried out in a cell *in vitro* and both nucleic acids are administered directly to the cell, or

2. the method is carried out in a cell *in vivo* and both nucleic acids are administered directly to the cell, or

3. the method is an *ex vivo* method wherein both nucleic acids are introduced into a cell and the cell is then administered to a subject.

The Examiner does not believe, however, that the specification enables a method of regulating expression of a *tet* operator-linked gene in a cell, wherein a first nucleic acid molecule comprises a nucleic acid molecule comprising the *tet* operator-linked gene, and a second nucleic acid molecule encodes a tTA, wherein the tTA comprises a Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eukaryotic cells, wherein:

[1.] "the cell is present in a subject *in vivo* and one or both [of] the nucleic acids are administered by different methods, or

[2.] wherein cells have integrated one nucleic acid in the genome at a site or at randomly and the second nucleic acid is administered by any method, or

[3.] a method wherein the first nucleic acid is present in a cell, the cell is administered to a subject and the second nucleic acid is administered to the subject by any method." Applicants respectfully traverse this rejection.

The claimed invention pertains to a method for regulating expression of a *tet* operator-linked gene in a cell of a subject using the tTA system. The method of the invention comprises introducing into the cell a first nucleic acid comprising the *tet* operator-linked gene, and introducing into the cell a second nucleic acid molecule encoding a tTA. The first and second nucleic acid molecules of the invention are not necessarily covalently linked to each other. The concentration of tetracycline, or analogue thereof, in the subject can be modulated to regulate expression of the gene of interest operatively linked to the *tet* operator.

Applicants maintain that the specification provides ample guidance to enable one of ordinary skill in the art to make and use the invention, as described in the response

filed March 8, 2002 and incorporated herein. The specification provides significant guidance regarding each component of the tTA system, describing specific preferred examples (see *e.g.* pages 12-14 and 25-27). In addition, the specification provides suggestions of alternative components which may be more applicable to certain situations, such as expression in a particular cell line. At page 30, lines 29-33, for example, the specification states that, "the tissue specificity of some promoters dictate that the tet operator sequence/promoter sequence fusion has to be designed with the particular application and cell line in mind following the teachings in this application using promoters customarily used for the cell line in question." These alternative components may readily be substituted using standard molecular biology techniques known to those skilled in the art. The specification still further describes how to operatively link a gene of interest to a *tet* operator sequence(s) (see *e.g.*, pages 14, 16 and 27). Moreover, the specification describes how to regulate transcription of a gene of interest using the transcriptional activator fusion protein (see *e.g.*, pages 29-31). As discussed in the specification, standard molecular biology techniques are used to construct the DNA and vectors of the invention.

Applicants further maintain that the specification fully enables one of ordinary skill in the art to use said invention in a gene therapy protocol. The specification describes in detail how to use the tTA system for regulation of a gene of interest in a gene therapy-based method for several disease states (see pages 32-36). Additionally, the specification cites, and incorporates by reference, several publications describing the state of the art in gene therapy (see page 32, lines 11-13). These references describe, for example, preferred vectors and delivery systems for particular target cell types. These publications thus provide extensive guidance to one of ordinary skill in the art at the time of filing the present application, regarding gene therapy constructs and methods.

Applicants reiterate that in order to meet the enablement requirement, it is not necessary that a patent specification include specific examples of every different embodiment encompassed by the claims. Moreover, the fact that some experimentation may be necessary to produce a gene delivery system to be used to deliver the nucleic acid molecules of the invention, does not constitute lack of enablement as long as the amount of experimentation is not unduly extensive. *Amgen Inc. v. Chugai Pharmaceutical Co., Ltd.,* 927 F.2d 1200, 1213 (CAFC 1991). A considerable amount of experimentation is permissible if it is merely routine, or if the specification provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. *In re Wands* 8 USPQ2d 1400-1407, 1404 (CAFC, 1988).

In the response filed March 8, 2002, Applicants presented numerous scientific publications which demonstrate that, contrary to the Examiner's assertion, the claimed invention has been used successfully in *in vivo* gene therapy protocols. Applicants provided scientific publications, including Dhawan *et al.* and Fishman *et al.* (references BW and BZ, respectively, submitted in the Information Disclosure Statement (IDS) filed previously), which described *in vivo* use of the tTA system by injecting plasmid DNA encoding the tTA system directly into muscle tissue. Dhawan *et al.* describe successful use of the claimed tTA system, wherein ***two separate plasmids encoding the tTA transactivator and a tet operator-linked luciferase reporter gene were co-injected into skeletal muscle of mice***. The injected plasmids were controlled both by oral and parenteral administration of tetracycline. Fishman *et al.* also describe successful use of the claimed tTA system wherein ***two separate plasmids encoding the tTA transactivator fused to a cardiac-specific promoter and a tet operator-linked luciferase reporter gene were co-injected into the hearts of rats.*** Fishman *et al.* demonstrate successful regulation of the Tet system *in vivo* where two non-covalently linked nucleic acid molecules are co-injected into muscle tissue of animals.

Applicants provide herewith post-filing evidence which shows that ***in vivo gene expression systems, including the tTA system, can be used successfully via a variety of delivery methods wherein the nucleic acid molecules are not covalently linked and are administered separately***. This additional evidence is in the form of journal articles, is provided herewith in a Supplemental IDS, and is summarized below:

1) Wilsey *et al.* (2002) *Gene Therapy* 9: 1492-1499 (see ref. G6 in Supplemental IDS). Wilsey *et al.* describe a gene therapy approach to study obesity, wherein leptin (the product of the *Ob* gene) is delivered to the hypothalamus of rats. As shown in Figure 1, two separate plasmids containing the *Ob* gene operatively-linked to a *tet* operator and the rtTA gene (the mutant version of the tTA gene of the present invention) were recombined into an adeno-associated viral (AAV) delivery system and injected into rat brains. Recipients were then examined for physiological effects, as shown in Figures 2 and 3. Recipient rats gained 51.7% less mass and ate 11.4% less food than the control groups as a result of the increase in leptin expressed from the tTA system.

2) Régulier *et al.* (2002) *Human Gene Therapy* 13: 1981-1990 (see ref. G3 in Supplemental IDS). Regulier *et al.* describe a lentiviral vector system for treatment of neurologic disorders, including Huntington's disease. The experimental design includes injecting rats with both a lentiviral vector containing the human ciliary neurotrophic factor (CNTF) operatively linked to a tet operator and a second lentiviral vector encoding tTA of the invention. ***Studies using the two separate vectors showed that 63.8% of the infected cells contained the two viral vectors.*** Furthermore, rats expressing the CNTF gene showed significant improvements in apomorphine-induced rotations.

3) Apparailly *et al.* (2002) *Human Gene Therapy* 13: 1179-1188 (see ref. G1 in Supplemental IDS). Apparailly *et al.* describe *in vivo* regulated therapeutic gene expression for treatment of rheumatoid arthritis using ***both a non-covalently and covalently linked tTA and a tet operator-linked gene*** (see Figures 5a and 1a,

respectively). As shown in Figure 5a, ***two separate vectors containing the rtTA (mutated version of tTA which has reverse binding properties) and IL-10 cDNA operatively linked to the tet operator*** were injected intramuscularly into DBA1 mice, a mouse model for rheumatoid arthritis. The mice were fed doxycycline to induce expression of IL-10, and assayed for IL-10 levels of expression following euthanization. The results show that IL-10 was expressed.

4) Pulkkanen *et al.* (2002) *Cancer Gene Therapy* 9:908-916 (see ref. G2 in Supplemental IDS). Pulkkanen *et al.* describe a gene therapy approach for the treatment of renal cell carcinoma using a combination therapy of *HSV-tk* (Herpes simples virus thymidine kinase) and *ES* (containing rat endostatin cDNA) adenoviruses. Adenovirus was injected intratumorally into nude mice, which were later evaluated for tumour growth. Mice receiving both the *HSV-tk* and *ES* adenovirus showed tumour regression or dormancy in comparison to the *HSV-tk* alone and *ES* alone injected mice.

5) Rose *et al.* (2002) *Gene Therapy* 9:1312-1320 (see ref. G4 in Supplemental IDS). Rose *et al.* describe a therapeutic approach for treating cystic fibrosis using both naked DNA and DNA/liposomes formulations comprising the CFTR gene (cystic fibrosis transmembrane conductance regulator). Rose *et al.* demonstrate successful administration of both naked DNA and DNA liposomes to murine airways by instillation.

6) Taniyama *et al.* (2002) *Gene Therapy* 9:372-380 (see ref. G5 in Supplemental IDS). Taniyama *et al.* describe a non-viral based gene therapy approach comprising plasmid DNA transfected using ultrasound with echo constant microbubble (Optison) to promote angiogenesis in the rabbit hindlimb ischemic model. Results shown in Figure 5 demonstrate that the combination of plasmid DNA (containing hepatocyte growth factor or HGF) and ultrasound with Optison, is effective in promoting angiogenesis *in vivo*.

Applicants thus submit that the specification and post-filing evidence show the successful regulation of gene expression by the claimed tTA system, wherein the nucleic

acid molecule comprising the tet operator-linked gene of interest is non-covalently linked to a nucleic acid molecule comprising a tTA *in vivo*. Furthermore, the claimed methods may be used for expression of a gene of interest at measurable levels and the level of gene expression can be manipulated by adjusting the amount of tetracycline present.

Applicants note that none of the cited references describe difficulties in obtaining two non-covalently linked nucleic acid molecules in one cell, such that both nucleic acids are incorporated into the cell, as set forth as a concern by the Examiner. Moreover, there are clearly numerous effective gene therapy protocols including direct injection of DNA, viral vector systems (*e.g.* adenovirus, AAV, and lentiviral vectors), liposome formulations, and ultrasound.

In view of the teachings in the specification and the general knowledge in the art, the specification has provided sufficient guidance to the ordinarily skilled artisan to make and use the invention, without undue experimentation. Moreover, the post-filing evidence further shows the success of the claimed methods. Accordingly, Applicants respectfully request that the rejection of claims 1-6 and 9 under 35 U.S.C. §112, first paragraph, be withdrawn.

Claims 10-14, 17, and 19 Are Free of Prior Art

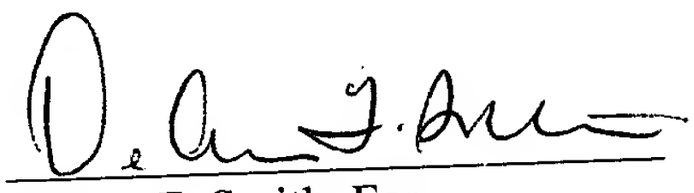
Applicants gratefully acknowledge the Examiner's indication that claims 10-14, 17, and 19 are free of the prior art of the record. Applicants respectfully request that the Examiner also indicate that claims 1-6 and 18 are free of the prior art.

SUMMARY

In view of the foregoing remarks, reconsideration of the rejections and allowance of all pending claims is respectfully requested.

If a telephone conversation with Applicants' Attorney would expedite the prosecution of the above-identified application, the examiner is urged to call Applicants' Attorney at (617) 227-7400.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

4. **(Amended)** The method of claim ~~1 or~~ 18, wherein the nucleic acid molecule encoding the tTA is integrated randomly in a chromosome of the cell.

5. **(Amended)** The method of claim ~~1 or~~ 18, wherein the nucleic acid molecule encoding the tTA is integrated at a predetermined location within a chromosome of the cell.